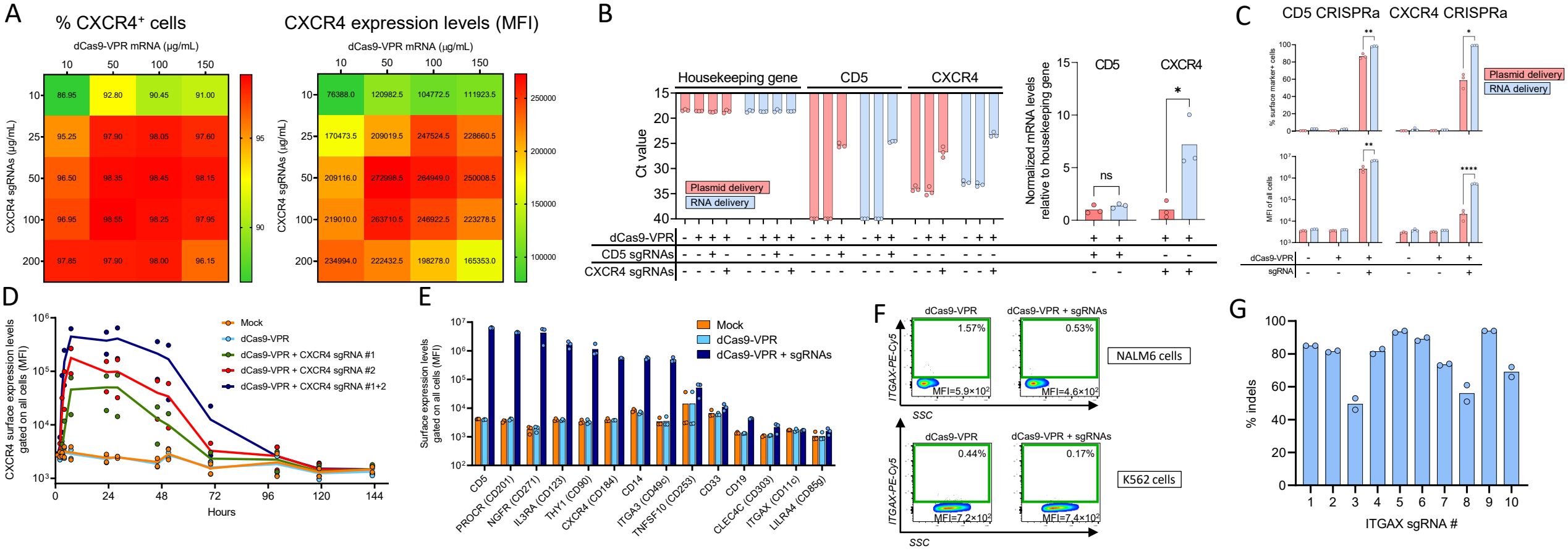
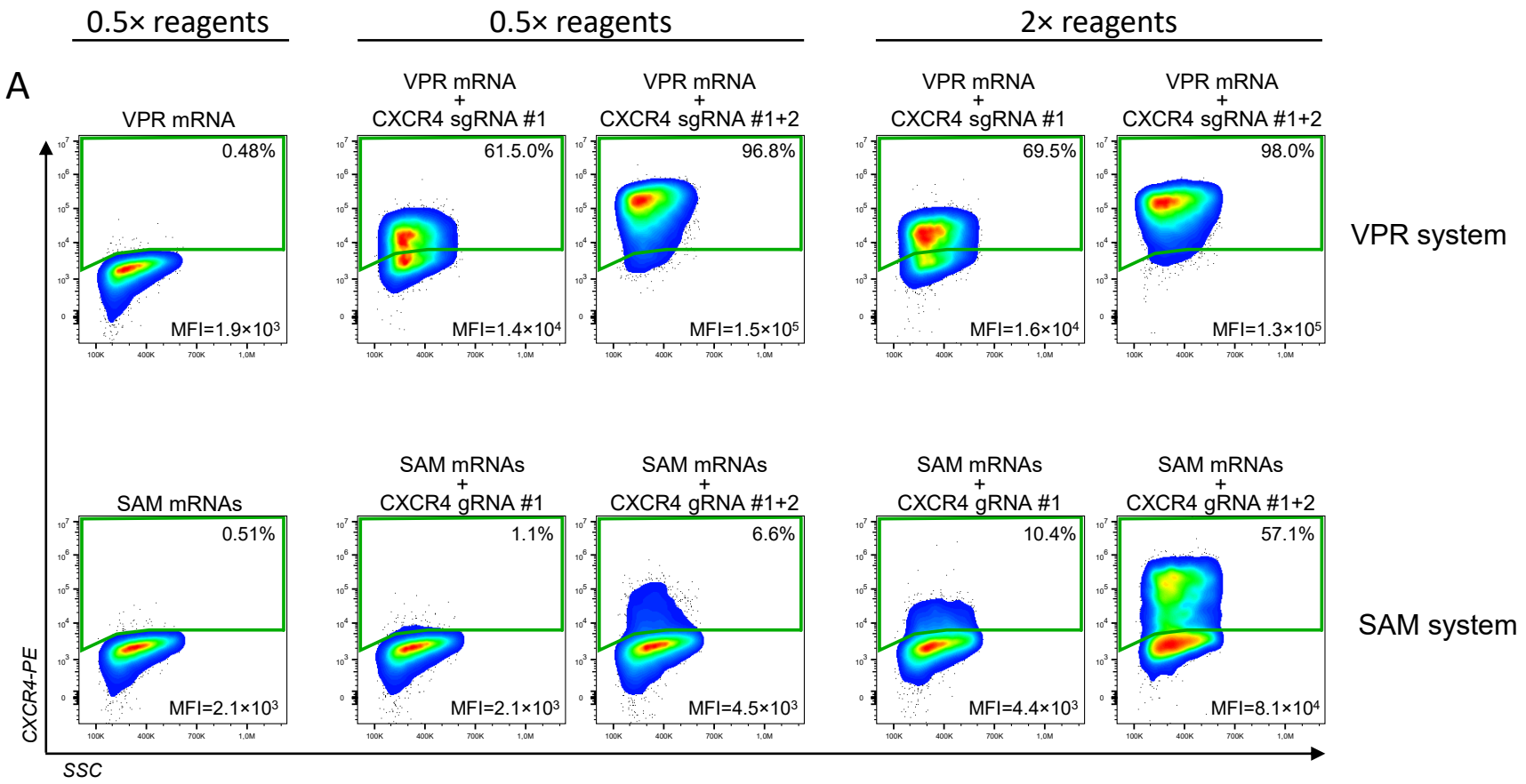
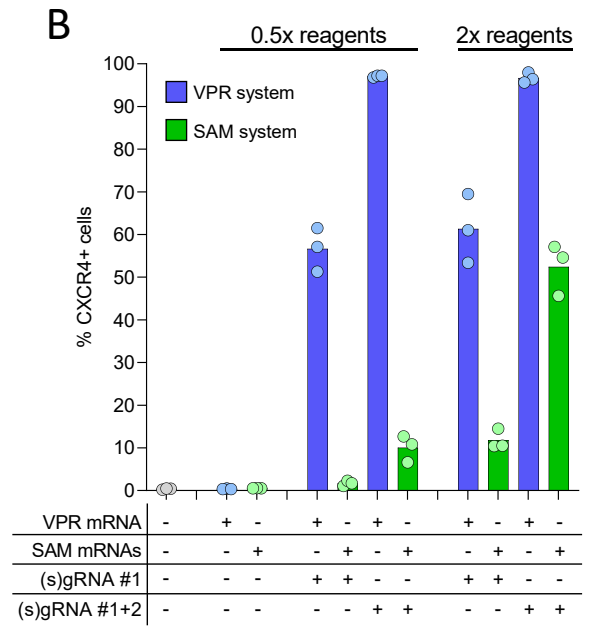
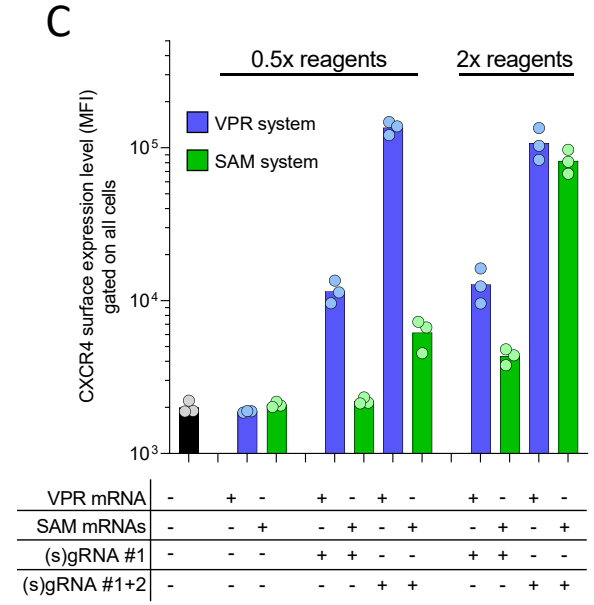


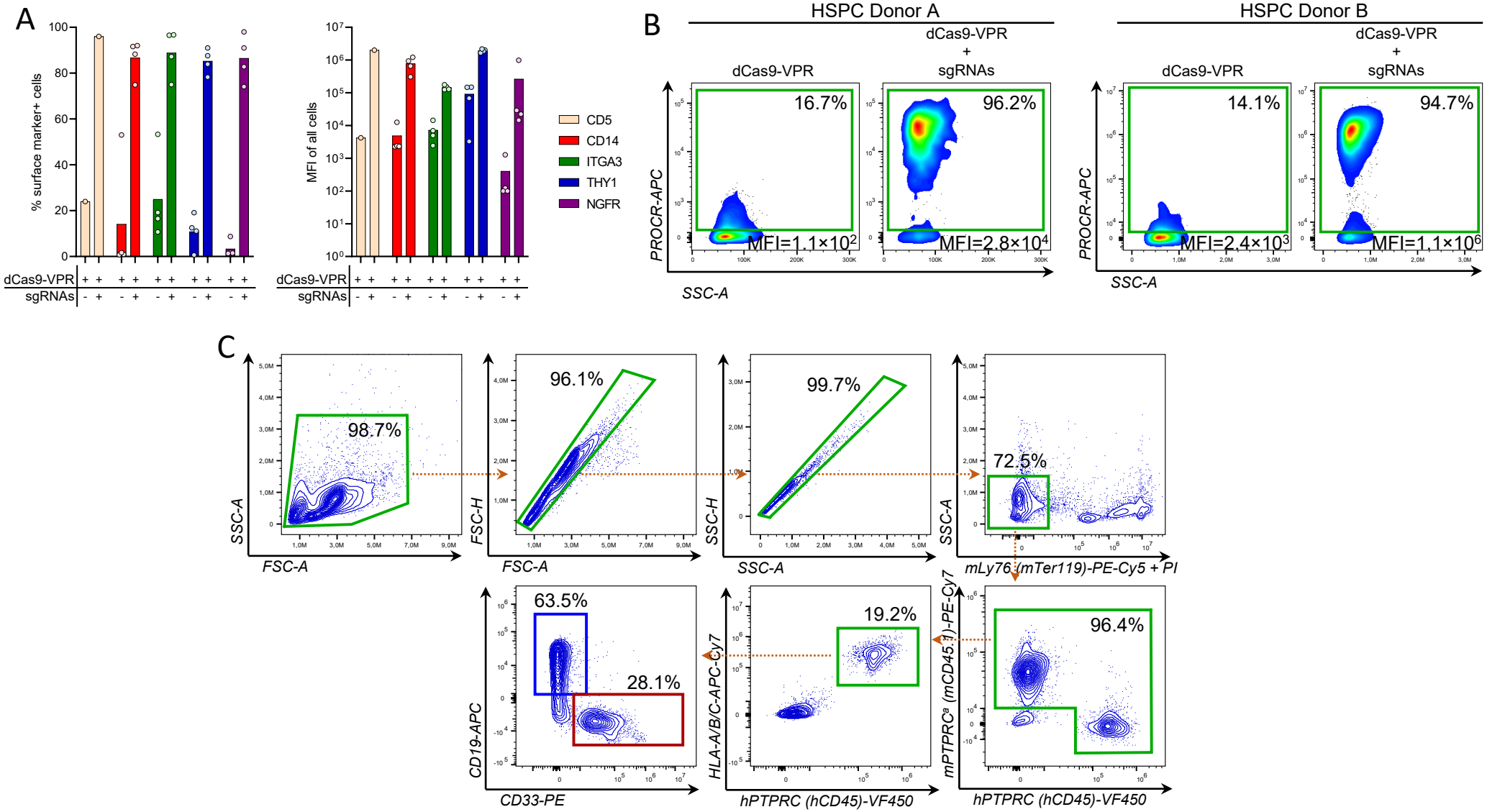
Supplementary Fig. 1. Investigating CRISPRa in the K562 cell line. A, Dose titration experiments determining optimal amounts of dCas9-VPR mRNA and sgRNAs. K562 cell were electroporated with varying amounts of dCas9-VPR mRNA and two sgRNAs targeting the TSS-region of *CXCR4*. Cells were analyzed by flow cytometry 24 hrs post-electroporation. The left heat map shows the percentage of CXCR4⁺ cells and the right heat map shows the MFI of all live cells. Numbers represent averages of two independent experiments. **B**, RT-qPCR analyses of target gene expression in K562 cells 24 hours after electroporation with *CD5* and *CXCR4* CRISPRa systems based on optimized conditions with either plasmid or RNA. Four sgRNAs were used for *CD5* and two sgRNAs were used for *CXCR4*. Since CD5 was not expressed in K562 cells, relative expression levels could not be calculated and instead, the left graph shows raw Ct values of *CD5*, *CXCR4*, and the house keeping gene RPLP0 (reference gene). The right panel compares relative target gene expression levels following CRISPRa using either plasmid- or RNA-based delivery. Here, mRNA levels of each target gene after CRISPRa were first normalized to RPLP0 and then normalized to the plasmid-based condition, with the latter set to 1. **C**, Flow cytometry-based analysis of cells with CRISPRa of *CD5* or *CXCR4* comparing plasmid- and RNA-based delivery as described above. Representative FASC plots are shown in Fig. 1B. The graphs show the percentage of surface marker-positive cells (upper panel) or the MFI of all live cells (lower panel) as measured 24 hrs post-electroporation. **D**, Time course experiment showing the CXCR4 MFI of all live cells measured by flow cytometry following electroporation with dCas9-VPR mRNA and two chemically modified sgRNAs. **E**, CRISPRa of 14 different target genes in K562 cells. Cells were analyzed by flow cytometry 24 hrs post-electroporation with dCas9-VPR mRNA and chemically modified sgRNAs (2-4 sgRNAs per gene). The surface marker MFI of all live cells is shown here, and the percentage of surface marker positive cells is shown in Fig. 1D. **F**, Further investigation of non-functional CRISPRa of *ITGAX*. The upper plot shows representative FACS plots (N=3) of ITGAX expression in NALM-6 cells 24 hrs after electroporation with dCas9-VPR mRNA and the same chemically modified sgRNAs used in K562 cells in Fig. 1D. The lower plot shows representative FACS plots (N=3) of ITGAX expression following electroporation with dCas9-VPR mRNA and six chemically modified sgRNAs targeting the region around the *ITGAX* TSS that are different from those used in Figure 1D. Percent ITGAX⁺ cells and MFIs of all live cells are indicated in the plots. **G**, Investigation of functionality of sgRNAs targeting the *ITGAX* TSS region. K562 cells were electroporated with nuclease-active Cas9 complexed with individual sgRNAs (RNPs), and indels at the *ITGAX* target sites were analyzed by Inference of CRISPR Edits (ICE; Synthego) four days post-electroporation. For all graphs, N = number of data points. All bars show mean values with individual data points plotted, * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001, ns P ≥ 0.05, student's *t*-test.



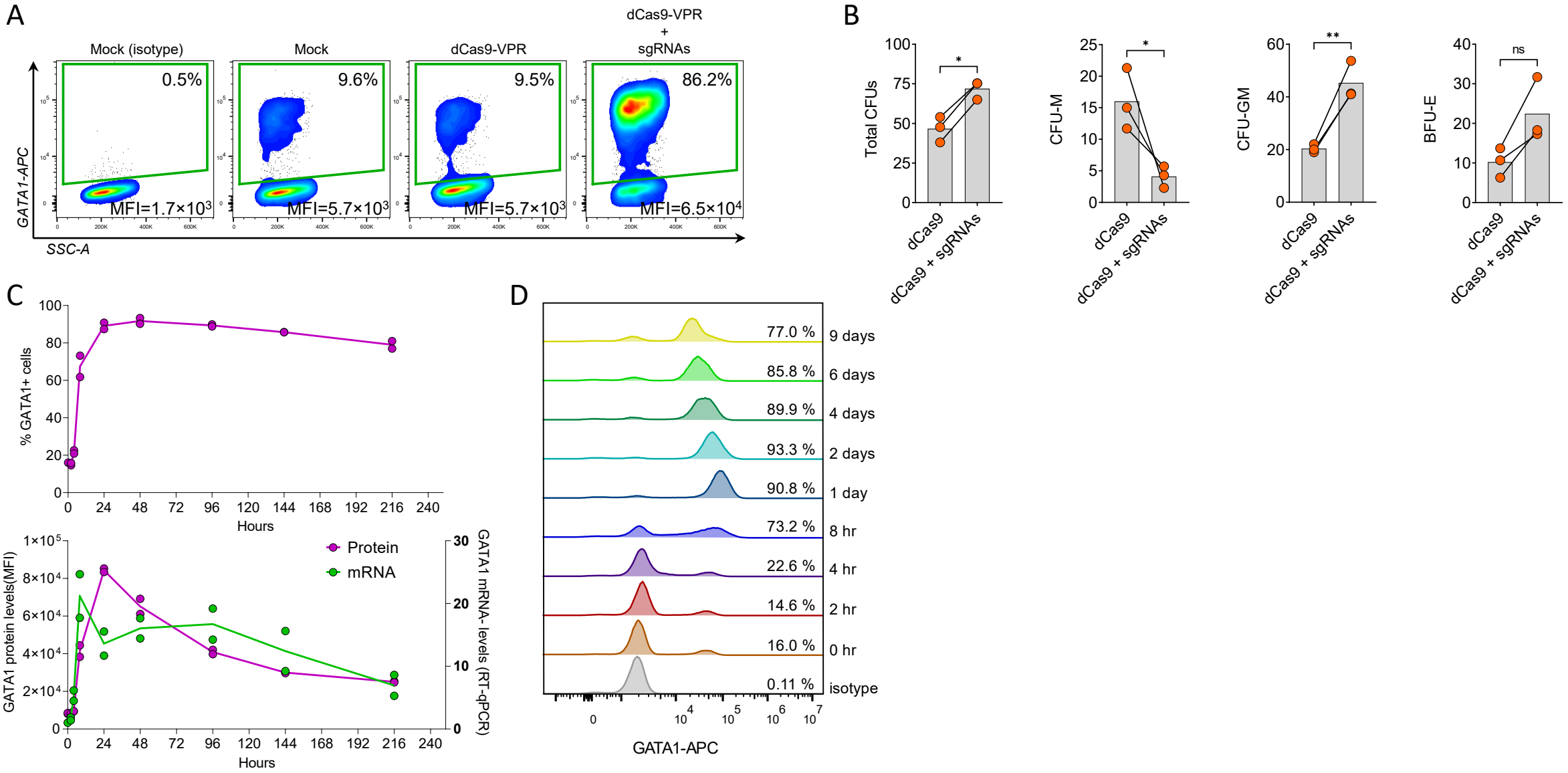




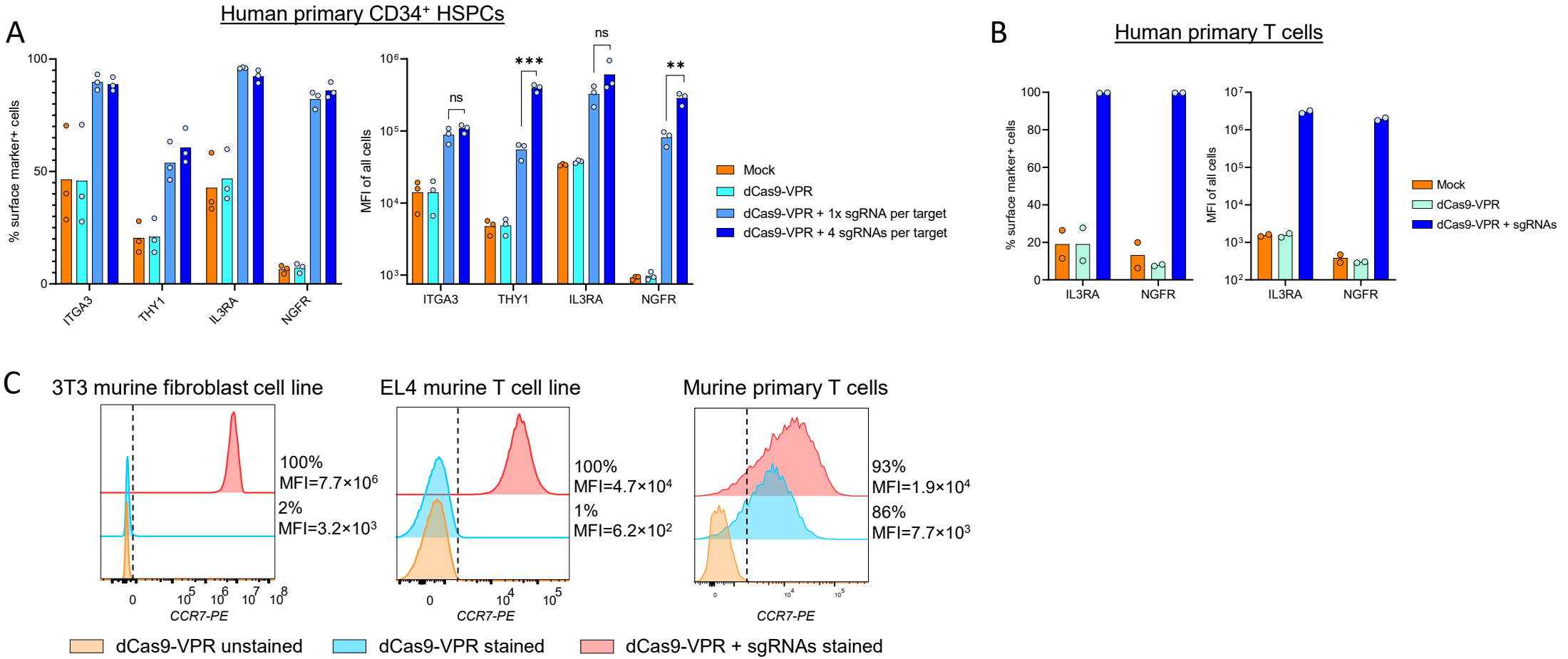
Supplementary Fig. 2. Comparing the activity of the VPR and SAM CRISPRa systems. To compare the VPR and SAM systems for CRISPRa of *CXCR4*, K562 cells were electroporated with dCas9-VPR mRNA (VPR mRNA) or dCas9-SAM mRNAs (SAM mRNAs, which are dCas9-VP64 mRNA and MS2-P65-HSF1 mRNA). For the SAM system, a two-part guide RNA (gRNA) system was used consisting of a crRNA and an MS2 aptamer-modified tracrRNA. For the dCas9-VPR system, a single guide RNA (sgRNA) was used. All guide RNAs were chemically modified to increase stability. For an equal comparison, the mRNAs were used at the same molar amounts (dCas9-VPR : dCas9-VP64 : MS2-P65-HSF1 = 1 : 1 : 1) and so were the guide RNAs (sgRNA : crRNA : tracrRNA = 1 : 1 : 1). To compare the systems at suboptimal efficiencies we performed experiments using half the optimal amounts (0.5× reagents) of dCas9-VPR+sgRNAs used throughout the study. To push the SAM system to optimal performance, we included a condition with double the amounts of the components (2× reagents). Cells were analyzed by flow cytometry 24 hours post-electroporation. **A**, Representative FACS plots show the percentage of cells positive for CXCR4 and the CXCR4 MFI of all live cells. **B**, The percentage of CXCR4⁺ cells and **C**, the CXCR4 MFI of all live cells are shown across triplicate samples. For all graphs, N = number of data points.



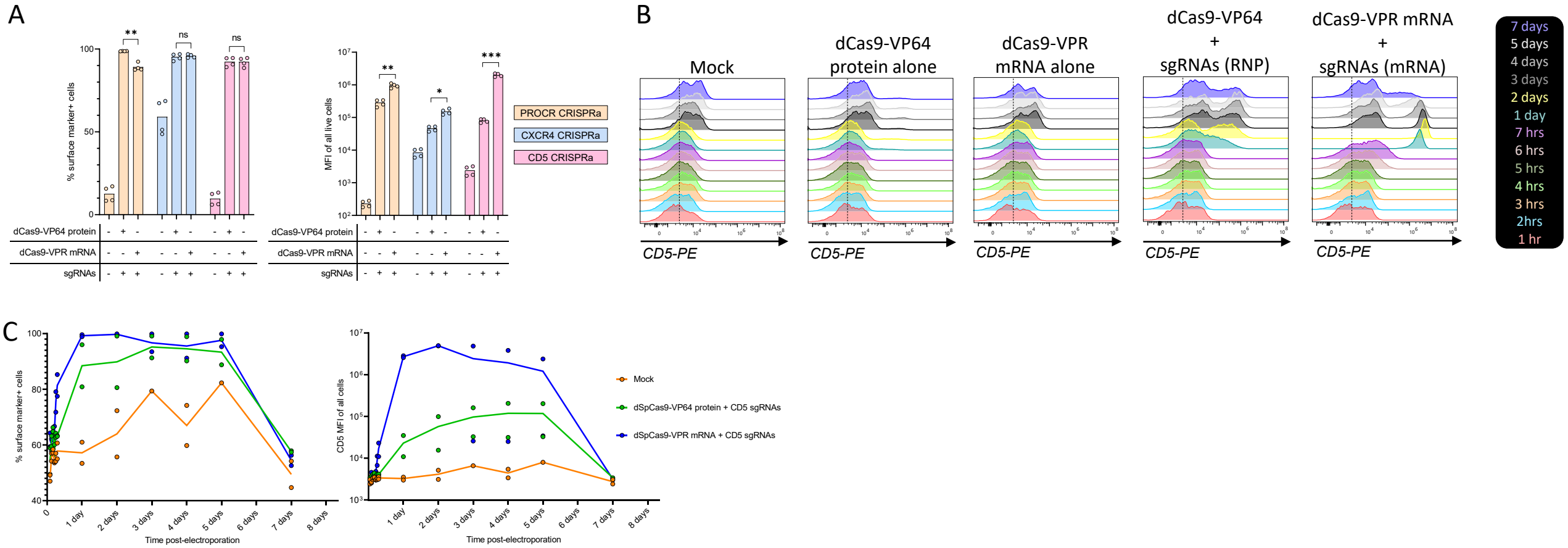
Supplementary Fig. 3. CRISPRa in human CD34⁺ HSPCs and confirmation of stem cell potential by transplantation and engraftment of cells in immunodeficient mice. A, Human CD34⁺ HSPCs were electroporated with dCas9-VPR mRNA and chemically modified sgRNAs (3-4 sgRNAs per gene). Percent surface marker-positive cells (left graph) and MFIs of all live cells (right graph) are shown. Representative FACS plots are shown in Fig. 3A. N = number of data points and all bars show mean values with individual data points plotted. **B**, Human CD34⁺ HSPCs from two cord blood donors (A and B) were electroporated with dCas9-VPR mRNA with or without three chemically modified sgRNAs targeting *PROCR*. 24 hrs post-electroporation, *PROCR* activation was confirmed by flow cytometry (note that the two donors were analyzed on different flow cytometers and the plots are not directly comparable). **C**, *PROCR*-activated cells and control cells not receiving sgRNAs were transplanted into irradiated immunodeficient NOG mice, 100,000 HSPCs per mouse, three mice per group. 20 weeks post-transplant, bone marrow of the transplanted mice was analyzed by flow cytometry for human chimerism and multilineage reconstitution (CD33⁺ myeloid cells and CD19⁺ B cells). Representative FACS plots show the gating strategy with the percentage of cells displayed within each gate. Human chimerism and multilineage repopulation results are shown in Fig. 3B.



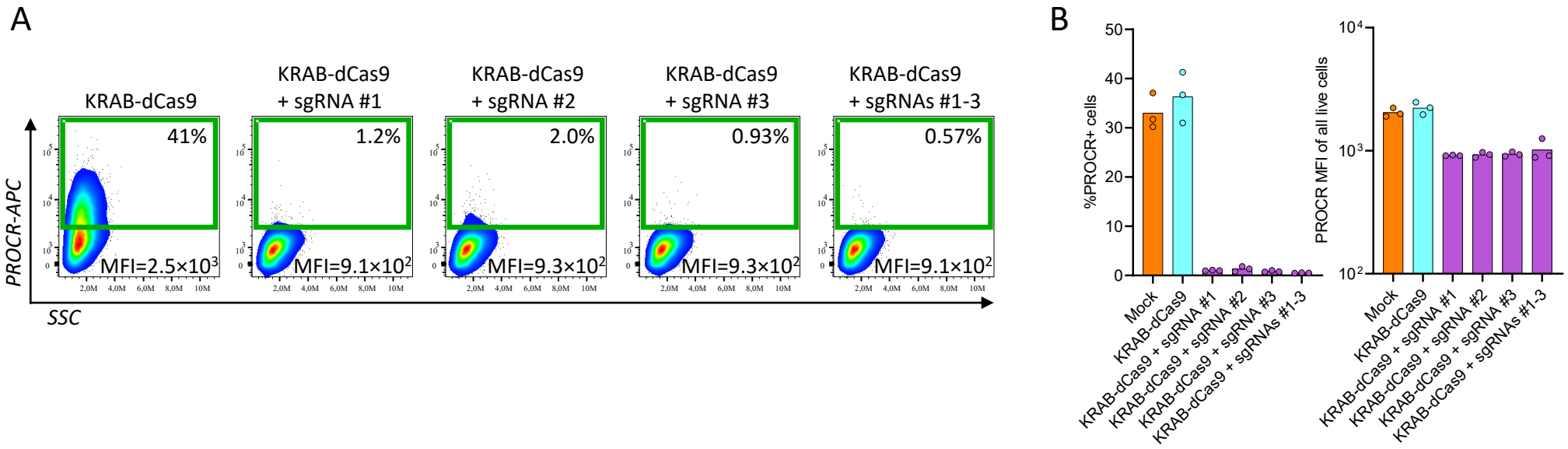
Supplementary Fig. 4. CRISPRa of *GATA1* in human CD34⁺ HSPCs changes hematopoietic differentiation trajectories. A, Human CD34⁺ HSPCs from three cord blood donors were electroporated with dCas9-VPR mRNA with or without four chemically modified sgRNAs targeting *GATA1*. 24 hrs post-electroporation, *GATA1* activation was assessed by intracellular flow cytometry. An isotype control antibody was used as a control. Flow plots show a representative donor. **B**, CD34⁺ HSPCs with or without *GATA1* CRISPRa were seeded into semi-solid methylcellulose media and 14 days after, colony-forming units (CFUs) were counted and analyzed by morphology and binned into the categories: macrophage (CFU-M), granulocyte/macrophage (CFU-GM), and burst-forming unit-erythroid (BFU-E). **C**, CD34⁺ HSPCs from two donors were electroporated with dCas9-VPR mRNA with or without four chemically modified sgRNAs targeting *GATA1*. At the indicated time points, *GATA1* activation was assessed by intracellular flow cytometry and RT-qPCR. The upper panel shows the percentage of cells expressing GATA1 protein and the lower graph shows overall protein levels (mean fluorescence intensity; MFI) and mRNA levels determined by RT-qPCR and presented relative to three housekeeping genes (ACTB, HPRT, and B2M) and normalized to time point 0. **D**, Representative FACS histograms for the intracellular GATA1 expression over time. For all graphs, N = number of data points and each data point represents a unique donor. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, ns $P \geq 0.05$, student's *t*-test.



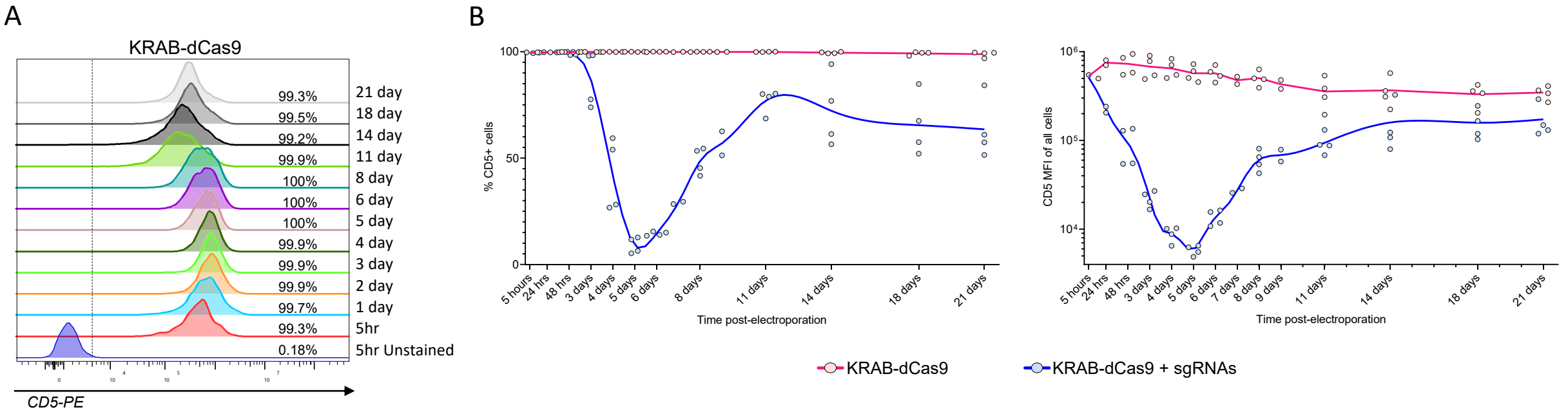
Supplementary Fig. 5. Multiplex CRISPRa in human HSPCs and single plex CRISPRa in human T cells and murine cells. A, Multiplexed CRISPRa of *ITGA3*, *THY1*, *IL3RA*, and *NGFR* in CD34+ HSPCs as analyzed by flow cytometry 24 hrs post-electroporation with Cas9-VPR mRNA and one or four sgRNAs targeting each gene. Left graph shows the percentage of cells positive for individual surface markers and the right graph shows the MFIs of all live cells for each surface marker. Representative FACS histograms are shown in Fig. 3D. **B**, CRISPRa of *IL3RA* or *NGFR* in activated human T cells. Flow cytometry was used to analyze the percentage of cells expressing the individual surface marker (left graph) or the MFIs of all live cells (right graph) 24 hrs after electroporation. Representative FACS plots are shown in Fig. 3E. **C**, Murine cell lines (3T3 fibroblasts and EL4 lymphocytes) and activated murine primary T cells were electroporated with dCas9-VPR mRNA and four sgRNAs targeting the murine *CCR7* TSS-region. Representative FACS histograms are shown with the vertical dashed line representing the threshold for CCR7 expression. For all graphs, N = number of data points and each data point represents a unique donor. All bars show mean values with individual data points plotted, * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001, ns P ≥ 0.05, student's *t*-test.



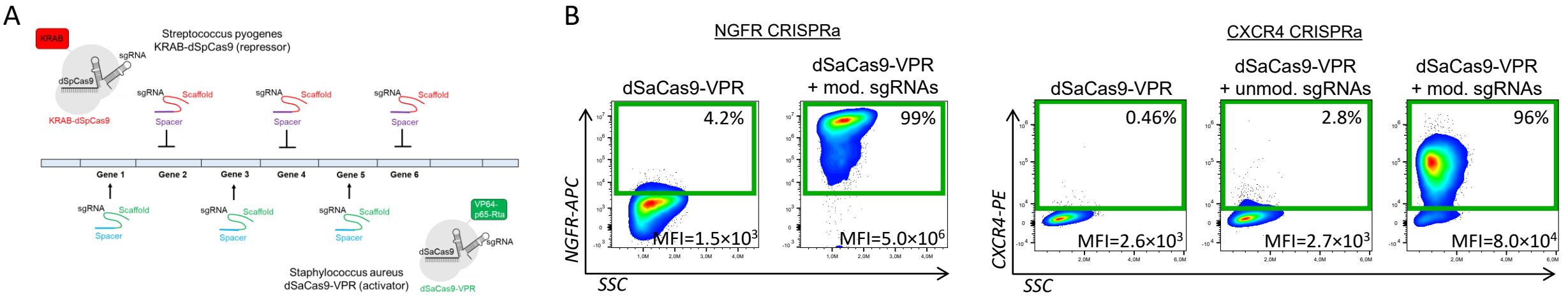
Supplementary Fig. 6. Comparison of protein and mRNA-based delivery of CRISPRa effectors. A, Data summarizing experiments in Fig. 4A comparing protein- and RNA-based delivery for CRISPRa across four different CD34⁺ HSPC donors. The left graph shows the percentage of cells expressing the target surface marker and the right graph shows the MFI of the target protein in all live cells. **B**, Time course of CRISPRa for protein- and RNA-based delivery for CRISPRa of *CD5* in CD34⁺ HSPCs. Representative FACS histograms show CD5 expression as analyzed by flow cytometry at the indicated time points. The colors of the histograms are color-coded with the time points indicated within the black bar. The dashed line indicates the threshold for CD5⁺ cells as determined by an unstained control. **C**, Time-course graphs summarizing the percentage of cells expressing CD5 (left) or the CD5 MFI of all live cells (right) following protein- or RNA-based delivery for CRISPRa of *CD5* in CD34⁺ HSPCs. Data is based on the FASC histograms shown in Supplementary Fig. 6B. For all graphs, N = number of data points with each data point representing unique donors. All bars show mean values with individual data points plotted, * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001, ns P ≥ 0.05, student's *t*-test.



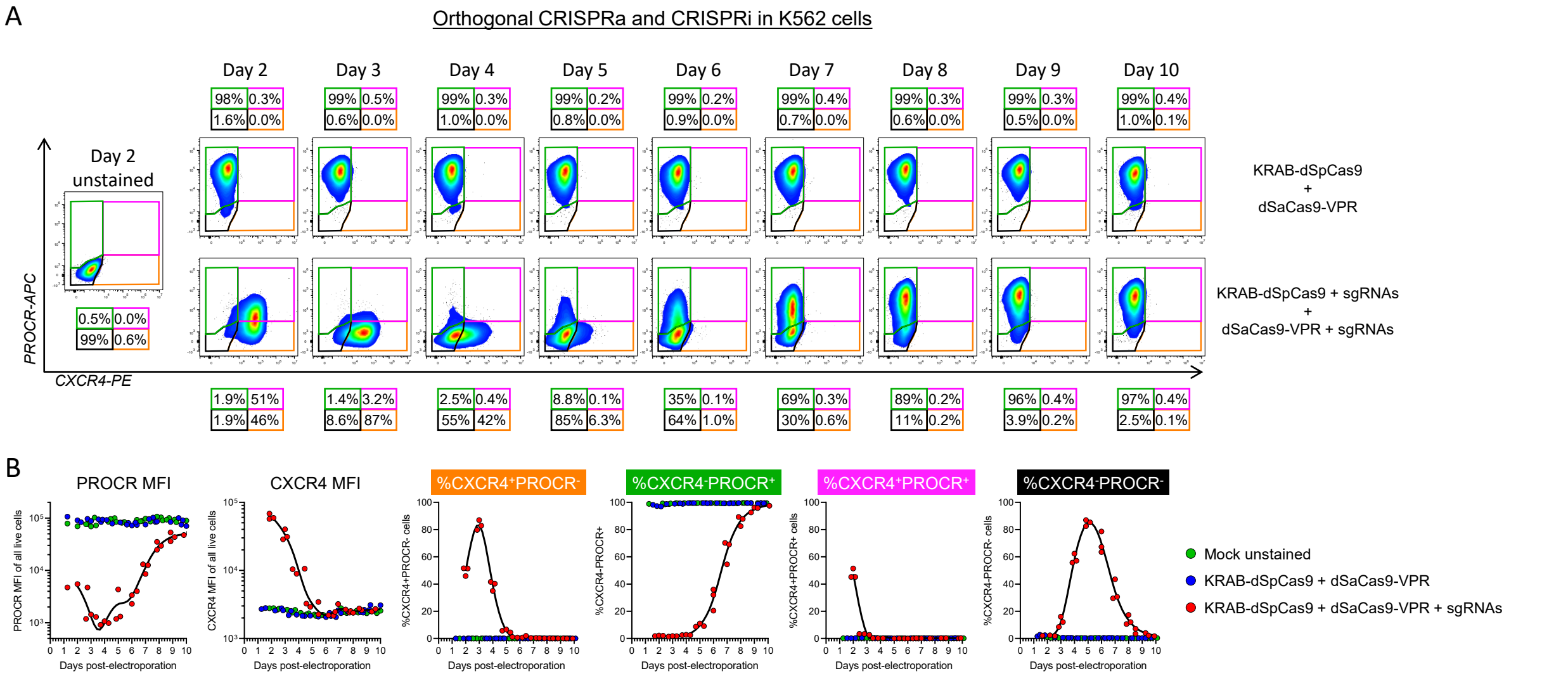
Supplementary Fig. 7. CRISPRi in the K562 cell line. A, K562 cells were electroporated with KRAB-dCas9 mRNA and individual or three chemically modified sgRNAs targeting the TSS region of *PROCR*. PROCR expression was analyzed by flow cytometry 72 hrs post-electroporation and representative FACS plots are shown with the percent PROCR⁺ cells shown in the gate and the MFI of all live cells. **B**, Graphs summarizing the percentage of PROCR⁺ cells (left) or the PROCR MFI of all live cells (right) for cells treated as in figure A. All bars show mean values with individual data points plotted.



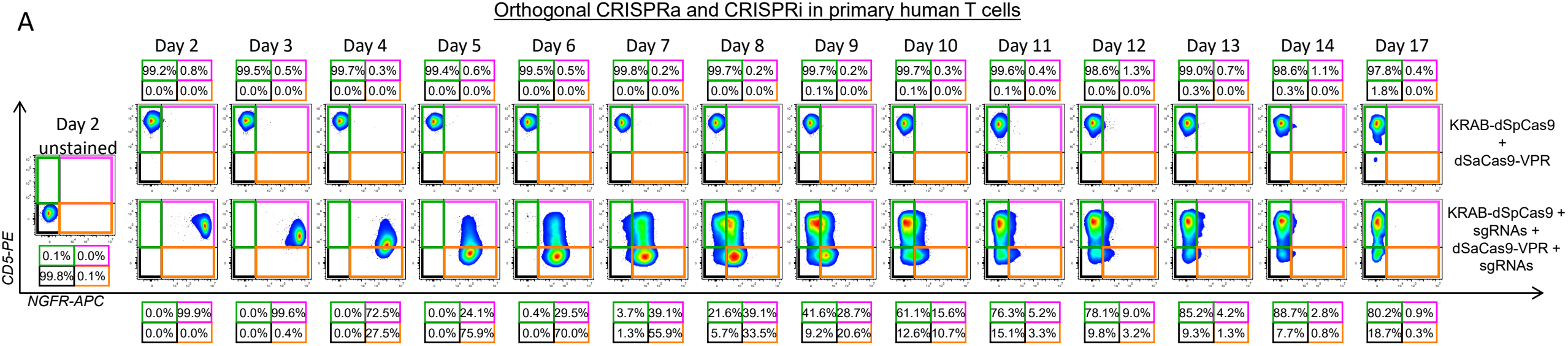
Supplementary Fig. 8. CRISPRi in primary human T cells. A, Supplementary figure for Figure 4B. Activated primary human T cells were electroporated with KRAB-dCas9 mRNA alone (shown in this figure) or with chemically modified sgRNAs targeting the TSS-region of *CD5* (shown in Figure 4B). CD5 expression was analyzed by flow cytometry at the indicated time points and FACS histograms show the percentages of $CD5^+$ cells. The bottom sample was not stained with the anti-CD5 antibody and was used to gate $CD5^+$ cells as indicated with the vertical dashed line. Cells were reactivated for three days by CD3/CD28 stimulation on day 8. **B**, Graphs summarize the percentage of $CD5^+$ cells (left) and the CD5 MFI of all live cells (right). N = 4 T cell donors with individual data points plotted.

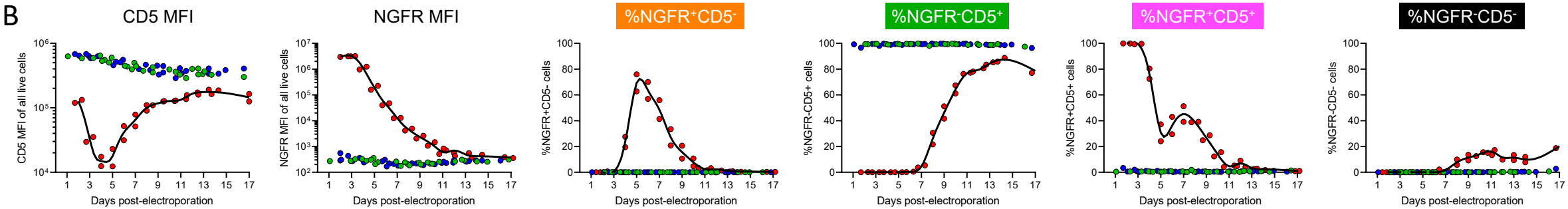


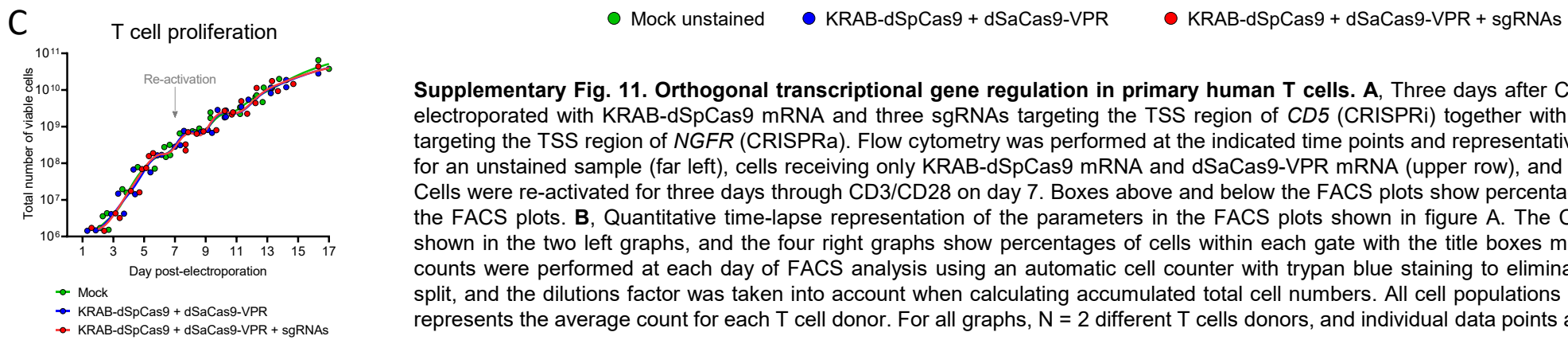
Supplementary Fig. 9. Principle of orthogonal CRISPRa and CRISPRi, and testing of staphylococcus aureus dCas9 for CRISPRa. A, Schematic figure showing orthogonal transcriptional regulation with streptococcus pyogenes dCas9 used for CRISPRi (KRAB-dSpCas9) and staphylococcus aureus dCas9 used for CRISPRa (dSaCas9-VPR). **B**, dSaCas9-VPR mRNA was produced by in vitro transcription and tested in K562 cells by electroporation alongside either three sgRNAs targeting the TSS region of *NGFR* or two sgRNAs targeting the TSS region of *CXCR4*. *CXCR4* sgRNAs were either unmodified (unmod.) or chemically modified (mod.) at both termini as described in the methods section. NGFR and CXCR4 expression was analyzed by flow cytometry 24 hrs after electroporation. FACS plots show the percentage of cells positive for the surface marker and surface marker MFIs of all live cells. N=1.



Supplementary Fig. 10. Orthogonal transcriptional gene regulation in K562 cells. A, K562 cells were electroporated with KRAB-dSpCas9 mRNA and three sgRNAs targeting the TSS region of *PROCR* (CRISPRi) together with dSaCas9-VPR mRNA and two sgRNAs targeting the TSS region of *CXCR4* (CRISPRa). Flow cytometry was performed at the indicated time points and representative FACS plots are shown (N=3) for an unstained sample (far left), cells receiving only KRAB-dSpCas9 mRNA and dSaCas9-VPR mRNA (upper row), and cells also receiving sgRNAs (lower row). Boxes above and below the FACS plots show percentages of cells in the color-matched gates of the FACS plots. **B**, Quantitative time-lapse representation of the parameters in the FACS plots shown in figure A. The PROCR and CXCR4 MFIs of all live cells are shown in the two left graphs, and the four right graphs show percentages of cells within each gate with the title boxes color-matched with the gate colors. For all graphs, N = 3, and individual data points are plotted.







Supplementary Fig. 11. Orthogonal transcriptional gene regulation in primary human T cells. A, Three days after CD3/CD28 activation, human T cells were electroporated with KRAB-dSpCas9 mRNA and three sgRNAs targeting the TSS region of *CD5* (CRISPRi) together with dSaCas9-VPR mRNA and two sgRNAs targeting the TSS region of *NGFR* (CRISPRa). Flow cytometry was performed at the indicated time points and representative FACS plots from one donor are shown for an unstained sample (far left), cells receiving only KRAB-dSpCas9 mRNA and dSaCas9-VPR mRNA (upper row), and cells also receiving sgRNAs (lower row). Cells were re-activated for three days through CD3/CD28 on day 7. Boxes above and below the FACS plots show percentages of cells in the color-matched gates of the FACS plots. **B**, Quantitative time-lapse representation of the parameters in the FACS plots shown in figure A. The CD5 and NGFR MFIs of all live cells are shown in the two left graphs, and the four right graphs show percentages of cells within each gate with the title boxes matching the colors of the gates. **C**, T cell counts were performed at each day of FACS analysis using an automatic cell counter with trypan blue staining to eliminate dead cells. During culture, cells were split, and the dilutions factor was taken into account when calculating accumulated total cell numbers. All cell populations were counted twice, and each data point represents the average count for each T cell donor. For all graphs, N = 2 different T cells donors, and individual data points are plotted.